

Studies on the Development of Echinoidea.

II. The Early Larva of *Echinocardium cordatum* and the Result of Crossing this Species with *Echinus esculentus*.

By

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With Plates 14 and 15.

THE results recorded in this paper were obtained during two months' sojourn at the Biological Station of the West of Scotland Marine Biological Association at Millport last summer. A preliminary account of the same has already been published in the 'Proceedings of the Royal Society' (16).

I have to record my thanks to Mr. Richard Elnhirst, Director of the Station, for the whole-hearted manner in which he aided my endeavours, and to Dr. James F. Gemmill, Vice-President of the West of Scotland Marine Biological Association, for the assistance he rendered me in providing me with pure cultures of diatoms, which were invaluable as food for the developing larvæ. To Prof. Graham Kerr, F.R.S., and to Dr. Agar, of Glasgow University, my best thanks are due for the loan of apparatus from the Zoological department of that University. And finally, my warmest thanks are due to my friend, L. W. Byrne, Esq., who re-drew my figures for me so as to make them suitable for publication.

The main object of my research was to test the distribution of paternal and maternal characters in the hybrid produced by crossing two species, in whose larvæ distinctly specific characters could be found.

A great deal of work has been done in crossing distinct

species of Echinoidea, and most contradictory results have been arrived at, as a short review of the principal papers on this subject will show.

Interest in the question of the character of the hybrid larvæ produced by crossing two species of Echinoidea was first awakened by two remarkable papers of Boveri (1, 2). In these papers, which record the same researches, he describes the effect of fertilising fragments of Sphærechinus eggs with spermatozoa derived from Echinus. He found that he obtained some hybrids of purely maternal type, others of mixed character, and some very small ones of purely paternal type. These last he attributed to the development of non-nucleated fragments of eggs which had been entered by spermatozoa of Echinus, since he had previously proved that non-nucleated fragments of eggs could develop into larvæ when entered by the spermatozoa of their own species. He therefore concluded that the nucleus alone was the bearer of heredity, since it could impress an Echinus-character on a fragment of a Sphærechinus egg.

This conclusion was attacked by Seeliger (17, 18), who confirmed Boveri's statement that enucleated fragments of eggs could be fertilised by sperm of the same species, but who denied that any hybrids figured by Boveri had been derived from such enucleated fragments, since, in a normal hybrid culture, larvæ of every type occur—from those showing a purely maternal character to those which closely approximate to the paternal type. Therefore he concluded that Boveri's inference that the nucleus alone was the bearer of the hereditary qualities was not sustained by his experiments. Seeliger also pointed out that Boveri had not properly described the typical form of the larva in each of the parent species, and that no valid conclusion as to the character of the hybrid could be drawn until this had been done.

Driesch (5) then took up the subject, and pointed out that in crosses of Sphærechinus and Strongylocentrotus Echinus the hybrids could be of purely maternal type in respect of (A) rapidity of development, (B) number of mesen-

chyme cells, (c) general form. But the first thorough investigation of the characters of the larvæ of the genera *Echinus* *strongylocentrotus* and *Sphærechinus* and of the hybrids produced by crossing these species was made by Vernon (23, 24). It must be borne in mind that it is possible to fertilise the eggs of *Sphærechinus* with the sperm of *Echinus* and *Strongylocentrotus*, but that eggs of *Echinus* or *Strongylocentrotus*¹ generally refuse to develop when fertilised by the sperm of *Sphærechinus*. Vernon came to the general conclusion that the predominance of paternal or maternal characteristics in the hybrid offspring was a question of the relative sexual ripeness of the male and female parents.

It may be incidentally remarked that the larvæ of *Echinus* and of *Strongylocentrotus* are not separable from one another by any clearly defined characters, but that those of *Sphærechinus* are sharply separated from the others by having the skeleton supporting each of the two post-oral arms in the form of a lattice-work consisting of several parallel calcareous rods joined to one another by numerous cross-bars. The skeleton of the post-oral arms of the other genera consists simply of single bars. It follows that the main feature relied on in determining which parent's influence predominates in the hybrid is the character of the skeleton of these arms.

Vernon's work was criticised in 1902 in a remarkably good paper by Steinbrück (20), in which he dealt with the cross between *Strongylocentrotus* and *Sphærechinus*. Steinbrück called attention to the fact that in their so-called distinctive characters the larvæ of both forms are variable; that in pure cultures of *Strongylocentrotus* larvæ are occasionally met with which possess two calcareous rods with occasional junctions between them in the post-oral arms, and that in pure cultures of *Sphærechinus*, larvæ are sometimes found in which the lattice-work is partly abolished in

¹ Driesch (5) records that 1 per cent. of *Strongylocentrotus* eggs develop when fertilised with *Sphærechinus* sperm.

the skeleton of the same arms; and further, that the hybrids between these two species are of a very variable character, so that from them a complete chain of forms can be selected leading from larvæ showing purely paternal to those showing purely maternal characters.

Steinbrück's work has not received the attention which was due to it. It was almost ignored by the next workers who occupied themselves with the subject—Doncaster and Herbst. Doncaster (4) admits that variations occur in the characters exhibited by pure cultures of *Strongylocentrotus* and *Sphærechinus*, but believes them to be relatively so infrequent that they may be disregarded, and he bases his estimate of the relative intensity of paternal and maternal influence exhibited in the character of the hybrid larvæ on the same criteria as those employed by Vernon. He arrives at the conclusion, however, that the influence of either parent—the father, for instance—does not vary with the ripeness, immaturity or staleness of the sexual products, but with the temperature of the water, for he found that he obtained the same results in December as in May if he artificially warmed the water used for the December cultures. This he explains by stating that warmed water causes the stronger larvæ to develop rapidly until they reach the point of development where further progress depends on food. Then they die and the weaker and more slowly developing larvæ survive them, and it is these that show the paternal influence most. In colder water the stronger larvæ develop more slowly, and hence are present at the time when the estimate is made (eight days after fertilisation).

Boveri (3) returned to the subject of the characters exhibited by the bastards produced by crossing *Strongylocentrotus*, *Echinus* and *Sphærechinus*. He maintains that the influence of the male parent is visible in the shape, skeleton, pigmentation, mesenchyme cells, and sometimes the size of the hybrid larva. To this Driesch replied (6), controverting all these points except the one concerning pigmentation.

Herbst, in a most elaborate paper (10), in which he deals

with the result of fertilising the eggs of *Sphærechinus* with the sperm of *Strongylocentrotus* and of *Echinus*, arrives at much the same results as Doncaster, but he admits that there is also a factor independent of temperature which determines the greater or less predominance of paternal characters, and this he finds in the varying character of the eggs. In a subsequent paper (11) he records the results of the experiment of initiating parthenogenesis in the eggs of *Sphærechinus* by treating them for a short period with valerianic acid and then fertilising them with the sperm of *Strongylocentrotus*. Under these circumstances he claims that he obtained a displacement of the development in the maternal direction, and he maintains that in a few cases he even obtained a larva maternal on the one side of the body and paternal on the other—a circumstance which he accounts for by supposing that the spermatozoon had entered the egg after the egg-nucleus had divided and had united with one of the two daughter-nuclei so formed.

Fischel (7) a little later dealt with the hybrids produced by crossing *Arbacia* and *Sphærechinus* and also *Arbacia* and *Strongylocentrotus*. (It is to be noted that he persistently and erroneously uses the term *Echinus brevispinosus* for *Sphærechinus granularis*). He arrived at results of generally the same character as those gained by Driesch; but he tries to show that the effect of foreign sperm entering the egg is to interfere with the normal distribution of pigment in the egg. He asserts that the spermatozoon can influence the rapidity of development, the form and size of the larva, the development of pigment, the skeleton and the histology of the cells.

Tennent (22) tried the experiment of crossing the American forms *Toxopneustes* and *Hipponoe*. The larvæ of *Toxopneustes* are like those of *Strongylocentrotus*. Those of *Hipponoe*, on the contrary, resemble those of *Sphærechinus*. The cross could be made by using *Hipponoe* as male or as female parent, but in either case the larvæ showed the influence of *Hipponoe* as evidenced by the "lattice-work" in the skeleton

of the post-oral arms. Tennent's most remarkable conclusion was that the *Hipponoe* influence in the hybrid could be decreased and the *Toxopneustes*' influence correspondingly increased by decreasing the alkalinity of the sea-water by the addition of a few drops of very dilute acid.

Hagedoorn (9) crossed two species of the same genus, viz. *Strongylocentrotus purpuratus* and *Strongylocentrotus franciscanus*, and arrived at the conclusion that the hybrid was in every case purely maternal in its character, but in a paper published a little while after (12) by Loeb, Redman King and Moore recording the results of hybridisation experiments between the same two species, these authors state that they are unable to confirm Hagedoorn's results, but arrive at the curious conclusion that certain characters appear in the hybrid whichever way the cross is made—are, in fact, a dominant over-correlative characters; so for instance the clavate aboral ends of body rods are dominant over the pointed form, the spherical form of larva over the pyramidal, the presence of the recurrent rod in the skeleton over its absence and so on.

The reader will gain from the preceding review an impression of results of a most unsatisfactory and contradictory character as the fruit of the work of all these experimenters. Having had considerable experience in rearing the normal larvæ of Echinoderms, it struck me that one principal cause of such discordant results was the great variability of the characters relied on as distinctive of the different species of larvæ.

I therefore looked about for "species" to experiment on whose larvæ were distinguished from one another by clear and unmistakable characters. Two possible cases presented themselves to mind: first the case of the species *Echinus esculentus* and *Echinus miliaris*, and second, the case of *Echinus esculentus* and *Echinocardium cordatum*.

With regard to the first case, it should be noted that the differences between the larvæ of the two species do not become clear until the larvæ are about a month old and have

developed all eight arms. These differences concern the arrangement of the ciliated epaulettes, and the appearance of a green pigment in the larvæ of *Echinus miliaris*; they were described by me in 1899 (15). If anyone unacquainted with the details of the normal development of Echinoderm larvæ were to read through the papers of all the workers on hybridisation he would never suspect that the *Echinopluteus* larva ever developed more than four arms; and in this circumstance alone a strong instance is afforded of the necessity of knowing the ordinary development of a species before we make it the subject of "experimental embryology." In his first paper Boveri actually described the larva of *Sphærechinus* as possessing only two arms! Whilst I was seeking an opportunity to begin work the questions of the result of hybridising *Echinus esculentus* and *Echinus miliaris* was taken up by Shearer, De Morgan and Fuchs, who published a preliminary note of the results of their work (19). In this note they maintain that the hybrid between these two species is of a purely maternal character with respect to whatever character be selected for examination; and they maintain that this maternal character is not altered by changing the acidity or alkalinity of the sea-water employed. This certainly is a remarkable result to obtain by hybridising two species of the same genus, and experiments which have been made at the Imperial College, the results of which will shortly be published, do not bear out the view of these authors.

The second case forms the subject of the present paper. Vernon (25) had already recorded the results of crossing the eggs of two species of *Echinocardium* with the sperm of *Arbacia*, *Echinus*, *Sphærechinus* and *Strongylocentrotus*, and he found that the hybrid larvæ were all of a purely maternal type. When the sperm of *Echinocardium* was used to fertilise the eggs of the other genera no result was obtained except in one instance with the eggs of *Echinus*. In this case one third of the eggs developed and produced larvæ of a purely maternal type. The great distinguishing feature of the larvæ of *Echinocardium*, which it shares with the larvæ

of other Spatangoidea, is the possession of an aboral process of the body supported by a special skeleton. This, according to Vernon, is only developed on the fifth day, but according to him it appears in all the hybrid larvæ, although in them it is shorter than in the normal larva. Tennent (21), in a short preliminary notice read before the International Congress of Zoology held in Boston in 1907, had already announced that he had crossed the eggs of *Moira*, a Spatangid, with the sperm of *Strongylocentrotus*. He gives no description of the hybrids, however, but in a later paper gives more details (23). He succeeded in fertilising the eggs of *Moira* with the sperm of *Toxopneustes*. He kept the larvæ alive for seven days. None of them developed the aboral spine. Nevertheless, he says that they were of the "maternal intermediate type." He also made the reciprocal cross and obtained similar "intermediate" larvæ! Some were of the purely maternal type and developed for twenty days, but the possibility is not excluded that these were developed from eggs fertilised by chance spermatozoa of their own species.

Since the results which I obtained differ markedly from those recorded by Vernon, and are in many respects different from those obtained by Tennent, it may be worth while to give some account of the methods employed. At Millport large numbers of both *Echinus esculentus* and of *Echinocardium cordatum* were available. The former species could be picked off the rocks a stone's throw from the laboratory at ordinary low tides; the latter species could be obtained by the bucket-full at low spring tides by digging in a sandy beach about half a mile from the laboratory. The tank-water in the laboratory was unpolluted by drainage of any kind, for the laboratory is situated over a mile and a half from the centre of the little town of Millport, and the full tidal current of the Firth of Clyde sweeps past the rocks on which it is built. In the water in the tanks the adults of both species live comfortably, but it is, nevertheless, inimical to the larvæ. The reason of this is to be found in the metallic

pipes through which it is poured into the tanks; it is only necessary to dip up the water from the Firth in an earthenware or glass vessel in order to provide the larvæ with a medium in which they can grow and flourish. The eggs of both species when shaken out of the ovaries are provided with a glassy chorion which makes fertilisation difficult, but if the eggs are allowed to stand in clean sea-water for an hour two before being fertilised this membrane disappears. As tested by controls in which the eggs of each species were fertilised by its own sperm, it was evident that the material used was of the healthiest kind. The eggs of *Echinocardium* fertilised with their own sperm ran through their complete larval development and metamorphosed into young urchins in great numbers, accomplishing the whole cycle in three weeks. A full account of the normal development of this species is reserved for another paper. In the case of *Echinus esculentus* the larvæ lived for three weeks, developed ciliated epaulettes and all eight arms, but as I had previously given a very full account of the normal development of this species I gave no further heed to the larvæ. My success in rearing the larvæ I attribute to the cultures of diatoms provided by Dr. Gemmill.

When the eggs of *Echinocardium* were treated with the sperm of *Echinus* a moderate number of hybrids were produced, although possibly not more than one egg in a thousand developed. These hybrids lived for eight days, but then, in spite of being surrounded by abundance of food, they died. When the eggs of *Echinus* were treated with the sperm of *Echinocardium* about an equal number (one in a thousand) developed, and the resulting larvæ were of a purely maternal type. When, however, the sea-water in which the fertilisation was accomplished was previously sterilised by being heated to 70° C. no single *Echinus* egg developed, and when it was found that a certain number of *Echinus* eggs would develop if allowed to stand in clean sea-water without the addition of any sperm at all, it became

obvious that the supposed hybrids were really normally fertilised eggs whose fertilisation had been brought about by the accidental and unsuspected presence of spermatozoa of *Echinus* in the water employed. One cannot help wondering whether some of the results recorded in the literature, cf. that of Driesch that 1 per cent. of *Strongylocentrotus* eggs develop when fertilised with the sperm of *Sphæerechinus*, may not be due to a similar source of error.

Once this source of error had been detected all further experiments in cross-fertilisation were made in sterilised seawater, and every adult urchin before being opened was carefully washed in fresh water in order to destroy any spermatozoa which might be adherent to the outside of the test. The instruments employed were also carefully sterilised after each urchin had been opened before another was taken in hand. New experiments under these conditions gave exactly the same results when *Echinocardium* eggs were fertilised with the sperm of *Echinus*, and I consequently feel complete confidence in the accuracy of the results so obtained, but when the eggs of *Echinus* were fertilised with the sperm of *Echinocardium* no single egg developed.

Several authors, notably Loeb (12) and Godlewski (8), have succeeded in fertilising the eggs of sea urchins with the sperm of animals belonging to distinct classes, even to distinct phyla of the animal kingdom of Crinoidea, Mollusca, etc. In the case of Mollusca it has been proved that the sperm nucleus does not unite with the egg nucleus, i.e. the chromatin which the former brings into the egg is not included in the first karyokinetic spindle. In the case of Crinoids, however, Godlewski asserts that sperm and egg nucleus do unite, and that the chromatin of both is included in the formation of the first spindle. In all such cases of heterogeneous fertilisation the larvæ are of a purely maternal type and show no trace whatever of the paternal influence, and therefore this kind of development is termed "Entwicklungs-erregung," on the supposition that it is due to a

chemical influence exercised by the entering spermatozoon on the egg analogous to the action of salts in producing artificial parthenogenesis and having no relation to the ordinary hereditary action of the spermatozoon. The method employed to bring about this anomalous development is to add a small quantity of dilute alkali to the water in which the heterogeneous fertilisation is effected. Godlewski found that the addition of 2.5 c.c. of $\frac{n}{10}$ solution of NaOH was most effective in producing results. I therefore tried the effect of fertilising the eggs of *Echinus* with the sperm of *Echinocardium* in vessels of sea-water, to which .5 c.c., 1.0 c.c., 1.5 c.c., 2.0 c.c. and 2.5 c.c. respectively of $\frac{n}{10}$ solution of NaOH had been previously added to every 100 c.c. of sea-water employed. In the mixture of 2 c.c. of $\frac{n}{10}$ NaOH to 100 c.c. of sea-water a few unhealthy granular blastulæ were observed. In none of the other mixtures did a single egg develop.

Now Loeb has shown (13) that it is possible to get the eggs of sea urchins to develop if they are treated for a very brief period with a weak solution of butyric acid, then washed in sea-water, and then placed for an hour or so in sea-water rendered hypertonic by the addition of a few cubic centimetres of $\frac{n}{10}$ solution of NaCl to every 100 c.c. of water. The effect of the butyric acid is to cause the eggs to form membranes closely similar to those formed by eggs when normally fertilised. Development then begins, but if the eggs are not subsequently placed in hypertonic sea-water they break up into spheres which resolve themselves into smaller spheres and the whole egg is thus reduced to a heap of granules. Now if the actual formation of a vitelline membrane be carefully watched it will be found that the first step in this process is the formation at the surface of the egg of a large number of minute spherules whose outer walls coalesce to form the membrane. Hence Loeb puts

forward the view that the chemical action of the spermatozoon in provoking development consists of two parts; first a process of cytolysis is set up in virtue of which a vitelline membrane is formed, but this process would lead to the destruction of the egg if it were not checked; accordingly a second chemical action sets in in virtue of which the first action is arrested. In artificial parthenogenesis the checking of the process of cytolysis is effected by the hypertonic sea-water.

I was curious to see what actually had happened to the *Echinus* egg when treated with the sperm of *Echinocardium*. Microscopical examination revealed the fact that the eggs had formed fertilisation membranes, showing that the spermatozoa had entered them, but that they had then undergone cytolysis. One such egg is shown in fig. 1, Pl. 14. The spermatozoa of *Echinocardium* were therefore able to produce a cytolytic action, but incompetent to check it when it went too far.

Here, then, the cause of the sterility of the cross has been unmasked.

Turning now to the experiments in which *Echinocardium* eggs were fertilised with *Echinus* sperm I shall divide what I have to say into three parts. First I shall describe carefully the normal development of *Echinus esculentus* up till the sixth day; then I shall describe that of *Echinocardium cordatum* up till the fourth day; and then I shall describe the development of the hybrids, which, although they lived for eight days, did not attain a greater degree of development than that reached by the normal larvæ in about five days.

(1) THE DEVELOPMENT OF *ECHINUS ESCULENTUS*.

On the morning following the fertilisation of the eggs, which had been accomplished in the previous afternoon, clear spherical blastulæ (Pl. 14, fig. 2) were seen swimming at the surface of the water. The vegetative pole was already marked by the appearance of the first mesenchyme cells, but they had not yet migrated into the blastocele. Soon the blastula

became flattened on the vegetative pole and the mesenchyme cells wandered inwards. The majority of them formed a ring round the periphery of the flattened surface, but some wandered up the sides of the blastula and even reached the animal pole. At this pole there is a tuft of specially long cilia. The invagination which forms the archenteron began at the vegetative pole, and the ring of mesenchyme cells became thickened at two spots diametrically opposite to one another, and in these thickenings the rudiments of the larval skeleton appeared as two trifold "stars." Each arm of each star grew out, the growth being caused by the deposition of calcareous matter by the mesenchyme cells which cling to the arm. One arm of each "star" grows upwards and outwards—this is the rudiment of the post-oral rod of the skeleton; one arm grows backwards towards the opening of the invagination or "blastopore" and is the rudiment of the future "body rod" of the skeleton, and one grows horizontally across and forms the "horizontal rod" of the skeleton.

The invagination now deepens and forms the archenteron and the larva is now termed a gastrula. From the apex of the archenteron a single transversely elongated vesicle is cut off, which is the rudiment of the cœlom. This stage, reached in two and a half days, is represented in Pl. 14, fig. 3. At the animal pole of the embryo a tuft of specially long cilia (*cil.*) is to be seen.

In the course of the next day the stomodæum makes its appearance as a shallow pit on one side of the anterior end of the larva. The side of the larva on which it appears becomes concave and constitutes the future ventral surface. The pit rapidly deepens and reaches the apex of the archenteron, with which it unites, and thus the alimentary canal is complete. The cœlomic sac has just previously divided into right and left halves. From the "star" on each side another branch is given off which extends upwards at the sides of the stomodæum and here causes a slight protrusion of the ectoderm, so that the outline of the anterior part of the larva becomes quadrate instead of being rounded as it was pre-

vously. This extra rod is the "antero-lateral rod" of the larval skeleton and the protrusion is the rudiment of the antero-lateral arm. Meanwhile the post-oral rod has pushed out a posterior protrusion of the ectoderm on each side just above the blastopore, now become the anus. This protrusion is the rudiment of the post-oral arm (called "anal arm" by Driesch and Herbst). Up till now the larva has been uniformly ciliated all over, with a tuft of specially long cilia at the anterior pole, but now the cilia become restricted to a ridge forming the edge of the concave ventral surface. This is the longitudinal ciliated band of the larva, and it runs along the edges of the protrusions which are the rudiments of the larval arms.

The tuft of specially long cilia becomes incorporated in the anterior border of this band.

A dorsal view of a larva in this stage is given in Pl. 14, fig. 4. As soon as the alimentary canal is complete spots of orange-red pigment appear just under the ectoderm all over the larva. The pigment is carried by wandering mesenchyme cells, which can be seen to migrate into the ectoderm and to discharge their pigment (which is probably of an excretory nature) to the exterior in the form of granules. During the next day the antero-lateral and post-oral arms grow longer and an adoral band of cilia (*ad.*, fig. 5) becomes well defined. This consists of two ridges of thickened epithelium lying in the ventro-lateral walls of the larval œsophagus belonging partly to the ectodermal and partly to the endodermal region of this, which carry long cilia. There is reason to believe that these cilia produce an outwardly directed current and that their function is to remove excess of food matter from the region of the mouth. At least if a living larva be watched, particles suspended in the water shoot out violently from the ventral side of the mouth. On the left side the madreporic pore-canal and pore are formed by the union of a dorsally directed up-growth from the left cœlomic sac and a slight in-pitting of the ectoderm. Constrictor muscles, which cause the œsophagus to exe-

cute peristaltic swallowing movements, are developed from the inner walls of the cœlomic sacs, where they rest against the œsophagus; and dilator muscles are represented by protoplasmic strings (*dil.*, Pl. 14, fig. 5) which join the anterolateral rods to the outer walls of the cœlomic sacs. A larva four days old viewed from the dorsal side is shown in fig. 5. When the larva has attained the age of six days it has increased greatly in size, and rudiments of the remaining four arms, viz. the two præ-oral and the two postero-dorsal, are visible as very slight protrusions of the ciliated band. Underneath the rudiments of the postero-dorsal arms is seen an accumulation of mesenchyme cells, in the centre of which a high power of the microscope reveals a minute calcareous spicule—the rudiment of the skeleton of the arm.

No such accumulation is seen beneath the rudiments of the præ-oral arms; their skeleton arises in a later stage, as a median dorsal spicule, situated above the œsophagus far from the arms, and the actual præ-oral rods are subsequent outgrowths from this spicule. This is important, because it proves that the outgrowth of the arm is not directly due to a mechanical push exercised by the growing arm rod, but must rather be due to a chemical influence emanating from the spicule. The aboral ends of the body rods become bent inwards in a crook-like form (*cr.*, fig. 6), often far better shown than in the specimen figured.

(2) THE DEVELOPMENT OF ECHINOCARDIUM CORDATUM.

The egg of *Echinocardium cordatum* is not more than two thirds the diameter of the egg of *Echinus esculentus*, and the blastula which develops from it and rises to the top about eighteen hours after fertilisation is correspondingly small. Moreover, it is not spherical, but is elongated along one axis more than along the other. It is not, however, regularly oval, but would be more correctly described as being of cylindrical shape, rounded at the ends. In fig. 7 (Pl. 14) one of these blastulæ is shown. It is a little older than the blastula of *Echinus* shown in fig. 2, and the mesenchyme is more

developed. The blastula in course of the next day becomes a gastrula, and this stage is shown in fig. 8. In the gastrula represented in this figure we see that the mesenchyme is arranged in a circle; it is termed the primary mesenchyme, because it is given off as in the *Echinus* embryo from the vegetative pole of the blastula before the invagination which forms the archenteron has begun. At two opposite points in this circle a special aggregation of mesenchyme is to be seen, and inside each aggregation there is already clearly to be made out the four-armed calcareous "star" which is the rudiment of the larval skeleton. Of the four arms, one is directed upwards towards the anterior pole of the larva; this becomes, as in *Echinus*, the antero-lateral rod. One is directed backwards towards the blastopore; this becomes the "body rod." One is directed horizontally and forms the horizontal rod; whilst the fourth is directed outwards into a very slight elevation as ectoderm. This elevation of the ectoderm is the first trace of the post-oral arm, and the arm of the star corresponding to it is the rudiment of the post-oral rod. This "rod" is double, i. e. it is represented by two rods parallel to each other. At the anterior pole of the larva is to be seen the group of specially long cells carrying specially powerful cilia (*cil.*) similar to what was described in the case of the larva of *Echinus*. The secondary mesenchyme, which is budded from the apex of the archenteron and becomes the loose connective tissue of the larva, is well seen in the figure. The gastrula rapidly develops into an *Echinopluteus* larva by the same stages as those described in the case of *Echinus*. A concave ventral surface becomes defined, and to the edges of this surface the cilia become confined. The anterior tuft of cilia becomes incorporated in the anterior border of this surface, and the post-oral arms also arise from its border. The stomodæum arises as a pit on the ventral surface, and in the larva shown in fig. 9 this is just touching the anterior apex of the archenteron, although the two cavities are not yet open into each other. The cœlom arises, as in *Echinus*, as a transversely

elongated outgrowth from the archenteron, which becomes nipped off and then divided into right and left vesicles. Spots of dark red pigment have been developed. But it is the skeleton which especially arrests our attention. The post-oral arms are already developed, and each is supported by two parallel rods connected with each other by numerous cross-bars. The antero-lateral arms have not yet appeared, but the antero-lateral rods are already formed—each a single calcareous rod. From each of these rods a branch (*r. r.*) projects backwards, this is the rudiment of the recurrent rod. The body rods are well-developed single bars. Between their aboral ends an accumulation of mesenchyme cells can be seen, in the centre of which a tiny calcareous star (*ab*) can be seen. This accumulation of cells represents the formative matrix of the skeleton of the aboral spike—a structure characteristic of the Spatangoid larva and not found in the larva of any regular Echinoid. During the course of the next day the antero-lateral arms sprout out and so does the club-shaped aboral spike. The recurrent rod has grown backwards parallel to the body rod which it now equals in length; this rod is usually vestigial in *Echinus esculentus*, but is shown on one side in the larva represented in fig. 6. But in *Echinocardium cordatum* the recurrent rods extend to the aboral pole and here fork; the dorsal fork unites with its fellow, whilst the ventral forks unite the ends of the body rods, and in this way a terminal ring is formed in every way comparable to the so-called “frame” at the aboral end of the *Sphærechinus* larva. The skeleton of the aboral spike (*ab*) consists of three slightly diverging rods connected with each other by cross-bars. At their anterior ends they join the terminal ring, which has just been described. One of these rods is dorsal and median, the other two are lateral. It follows that the aboral spike possesses a most complicated skeleton. The apex of the aboral spike is covered with a crest of long-ciliated cells (*cil.*, figs. 10 and 11). During the course of the next day the rudiments of the postero-dorsal arms make their appearance and in each there is a tiny star,

the rudiment of its skeleton (*p.d.*). The cœlomic sacs, which in the previous stage lay at the sides of the œsophagus, now begin to grow back along the sides of the stomach and on each side a madreporic pore is formed. Whether this duplicity of the madreporic pore is constant or not I have not yet been able to determine; it is, at any rate, very frequent. A larva in this stage of development is represented in fig. 11. Further than this stage it is not my intention to pursue the history of the development. The larva of *Echinocardium* has in four days reached the same stage of development as that attained by the larva of *Echinus esculentus* in six days.

Let us now review the differences between the two types of larvæ. Leaving out the question of the duplicity of the madreporic pore, which may turn out to be a most important character but which cannot be used at present—we find that the larva of *Echinocardium* differs from that of *Echinus*—

(1) In possessing an aboral spike supported by a complicated skeleton, whilst at the aboral pole of the *Echinus* nothing of this kind is to be seen.

(2) In possessing “latticed” bars instead of single rods as supports for the post-oral arms. (Exceptionally in *Echinus* a second rod can appear in the post-oral arm. A trace of such rod is represented in fig. 4. I have never seen cross-connections between the two, but I am informed that these also can appear as a rare exception.)

(3) In possessing well-developed recurrent rods instead of only vestiges of such rods.

(4) In possessing brownish-red instead of orange-red pigment.

(5) In its more rapid development.

We shall now see how far these characters are represented in the hybrids.

DEVELOPMENT OF EGGS OF *ECHINOCARDIUM CORDATUM* FERTILISED WITH THE SPERM OF *ECHINUS ESCULENTUS*.

The earlier course of the development of *Echinocardium* eggs fertilised with the sperm of *Echinus*

esculentus is very similar to that undergone by these eggs when fertilised with the sperm of their own species. Fig. 12 shows the appearance of the free-swimming blastula twenty-four hours fertilisation. In this specimen the vegetative end is rather broader than the animal end, whereas in the blastula of *Echinocardium* represented in fig. 7, the reverse is the case, but this difference between the hybrid and normal blastula is not constant. By the end of the second day the blastula is converted into a gastrula such as is shown in fig. 13. The rate of development of the hybrid, however, varies with the specimens used as parents. In fig. 14 a hybrid larva of the same age is represented, which, however, belonged to a different culture, and we can see that it has attained the stage where the calcareous stars have been formed and where the cœlon is already grooved off from the archenteron. If from the same specimen eggs are taken some of which are fertilised with sperm of its own species and some with the sperm of *Echinus esculentus*, then the hybrids will always develop more slowly than the normal larvæ. As development proceeds the hybrids fall more and more behind the normal larvæ. In fig. 15 a hybrid four days old is represented. In this specimen the post-oral arms are well developed but the stomodæum has not joined the œsophagus. The bars supporting these arms are "latticed," but the cross bars are comparatively few. The antero-lateral bars are there but the antero-lateral arms are not yet developed. As shown in fig. 16, however, hybrids four days old may be more advanced in development. In the specimen shown in this figure the alimentary canal is complete and the post-oral arms are longer than in the specimen shown in fig. 15. But the supporting bars of these arms are single rods for the greater part of their length, although a second short rod accompanies the first at its base, as it does as a variation in *Echinus esculentus*. Both larvæ agree in the total absence of any indication of the aboral spike or of its skeleton.

When we turn our attention to the hybrid larvæ five days

old which are shown in figs. 17 and 18 the same total absence of an aboral spike or any indication of its skeleton strikes us. The antero-lateral arms are now developed. In the larva represented in fig. 17 the skeleton of one post-oral arm consists at its base of no less than four parallel rods; more distally a curious vestige of latticing is seen in the form of short rods accompanying the main rod and each connected with it by a transverse bar. The ends of the body rods are in-bent in a crook-like fashion such as occurs in the normal larva of *Echinus esculentus*. The larvæ represented in fig. 18 has an almost normal Echinocardium skeleton in each of the post-oral arms, but it is absolutely devoid of the aboral spike. In fig. 19 a hybrid six days old is represented; it shows much the same features as those shown by the five-day hybrid represented in fig. 18, only the arms are better developed and the crook-like in-bending of the aboral ends of the body rods is very marked. We notice also that a recurrent rod is well developed. In the seven-day larva shown in fig. 20 one of the antero-lateral arms has been absorbed—a phenomenon which often occurs with normal larvæ which are not quite healthy. The skeleton of this larva shows hardly any trace of the maternal influence; it is almost purely of the paternal type.

None of the hybrids lived longer than eight days although they were supplied with abundant food. Three of these eight-day larvæ are represented in figs. 21, 22 and 23. That shown in fig. 21 has a skeleton almost purely paternal in character; the only hint of maternal influence is to be seen in the thorns besetting the single rods which constitute the skeleton of the post-oral arms. One striking maternal character is, however, shown in the duplicity of the madreporic pore. The larva shown in fig. 22 has a double rod in each post-oral arm, but on one side the two rods are fused into one for the middle of their length, whilst on the other they are widely separated from one another and connected by a few cross-bars. Finally in the remarkable larva shown in fig. 23 there is a skeleton of a purely maternal type, and the aboral spike

is typically developed. The larva is distorted, however, since the oral lobe containing stomodæum is, as it were, twisted round on the body, and the result of this twist on the disposition of the skeletal rods is at first not a little puzzling. Care, however, enables us to recognise all the constituent parts of the maternal skeleton.

To sum up: The hybrid produced by fertilising the eggs of *Echinocardium cordatum* with the sperm of *Echinus esculentus* follows the mother in the character and distribution of the pigment: it is much smaller than larvæ of either the paternal or maternal species; it almost always follows the father in the total absence of the aboral spike and of its supporting skeleton, since in only one hybrid out of the hundreds examined was the aboral spike formed. In the skeleton of the post-oral arms the hybrid may be of the paternal type, of the maternal type, or of an intermediate character. In the inbending of the aboral ends of the body rods the hybrid follows the father.

The most important of these results is undoubtedly the total inhibition in the vast majority of cases of the formation of the aboral spike in a larva developed from a *Spatangid* egg, and the formation of a larva with a rounded aboral end and in-bent body rods, recalling in these features the *Echinus* larva. When we recollect that according to Shearer, De Morgan and Fuchs the crossing of two species of the genus *Echinus* results in the production of larvæ of the maternal type, no matter what feature is considered, it is not a little remarkable to find the paternal influence so strong in a cross between two species belonging not only to different genera but to different orders, species which must have diverged from a common ancestor at the beginning of the secondary epoch many millions of years ago.

If the attempt be made to explain the absence of the aboral spike as a mere concomitant of the feeble development of the hybrid, a glance at the figures of normal *Echinocardium* larvæ will be sufficient to refute this suggestion. We see there that the aboral skeleton and its formative

mass of mesenchyme can be detected in the normal larva before the antero-lateral arms have developed at all, and in many of the hybrids the antero-lateral arms are well developed. The absence of the aboral spike is therefore not a mere consequence of stunted growth, but is due to paternal influence. Moreover, it is not possible to reconcile the facts just recorded with any theory of dominance such as that put forward by Loeb, Redman, King and Moore.

If any clear meaning be attached to the word "dominance," it must signify that there is a certain factor which may be present or absent in a germ-cell, but which, when it is present, produces in the resulting embryo a certain character. Now if we take the question of the skeletal rods supporting the aboral arms, these are normally single in *Echinus* and latticed in *Echinocardium*. When these two species are crossed we do not always find either single or latticed bars, but we find, as a matter of fact, every intermediate condition of affairs. To alter the significance of the word "dominance" in such a way as to make it include phenomena like these is, in my opinion, to empty it of all its meaning.

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June 25th, 1912.

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EXPLANATION OF PLATES 14 AND 15,

Illustrating Prof. E. W. MacBride’s paper entitled “Studies on the Development of Echinoidea, Part II.”

LIST OF ABBREVIATIONS EMPLOYED.

ab. Skeleton of aboral spike of Echinocardium larva. *ad.* Adoral ciliated band. *a.l.* Skeleton of antero-lateral arm of larva. *an.* Anus. *b.r.* Body-rod of skeleton of larva. *calc.* First rudiment of skeleton of larva. *cil.* Anterior tuft of long cilia and posterior tuft of long cilia in Echinocardium larva. *cæ.* Cœlomic sac. *cr.* In-bent crook of body-rod in skeleton of larva of hybrid and of Echinus. *dil.* Dilator muscles of cœlomic sac. *f.* Fertilisation membrane. *h.r.* Horizontal rod of larval skeleton. *int.* Intestine. *l.p.c.* Left posterior cœlom. *mad.* Normal left madreporic pore. *mad.*¹ Right madreporic pore of Echinocardium larva. *mes.* Primary mesenchyme. *œs.* Œsophagus. *p.d.* Rudiment of skeleton of postero-dorsal arm. *pp.o.* Rudiment of pre-oral arm. *p.o.* Skeleton of post-oral arm. *r.p.c.* Right posterior cœlom. *st.* Stomach. *stom.* Stomodæum.

[All the figures were drawn from living larvæ with the help of the camera lucida, and all the larvæ figured are represented with a uniform magnification of 200 diameters so that their relative sizes can be seen.]

PLATE 14.

Fig. 1.—Egg of Echinus esculentus treated with the sperm of Echinocardium cordatum and examined twenty-four hours afterwards. *f.* Fertilisation membrane.

Fig. 2.—Blastula of Echinus esculentus twenty hours old. *mes.* Primary mesenchyme in the act of being formed.

Fig. 3.—Late gastrula of Echinus esculentus two and a half

days old. *cæ.* Cœlomic sac just separating from archenteron. *b. r.* Body-rod. *h. r.* Horizontal rod, and *p. o.* post-oral rod as three branches of trifold calcareous star, the rudiment of the larval skeleton.

Fig. 4.—Larva of *Echinus esculentus* three days old viewed from the dorsal side. *a. l.* antero-lateral rod of skeleton. *cæ.* Cœlomic sacs. *æs.* Endodermal part of œsophagus. *stom.* Stomodæum. *p. o.* Post-oral rod of skeleton (notice the supplementary rod on right side).

Fig. 5.—Larva of *Echinus esculentus* four days old viewed from the dorsal surface. *ad.* Thickening of epithelium carrying adoral ciliated band. *dil.* Dilator muscles of cœlomic sac and of œsophagus attaching these structures to the antero-lateral rod of the larval skeleton. *mad.* Madreporic pore.

Fig. 6.—Larva of *Echinus esculentus* six days old viewed from the dorsal surface. *cr.* Inbent crook at aboral end of body-rod. *p. d.* Accumulation of mesenchyme cells, the formative tissue of skeleton of postero-dorsal arm. *p. r. o.* Rudiment of præ-oral arm. *r. r.* Vestige of recurrent rod of skeleton.

Fig. 7.—Blastula of *Echinocardium cordatum* twenty-four hours after fertilisation. *mes.* Primary mesenchyme being formed.

Fig. 8.—Gastrula of *Echinocardium cordatum* one and a half days old. *calc.* Rudiment of skeleton. *cil.* Anterior tuft of long cilia. *mes.* Secondary mesenchyme being budded from the apex of the archenteron.

Fig. 9.—Larva of *Echinocardium cordatum* two days old viewed from the dorsal surface. *ab.* Calcareous star, the rudiment of the skeleton of the aboral spike embedded in a mass of formative mesenchyme. *p. o.* Latticed skeleton of post-oral arm. *æs.* Endodermal œsophagus which is in contact with, but which has not yet opened into, *stom.* the stomodæum. *r. r.* Recurrent rod of the skeleton.

Fig. 10.—Larva of *Echinocardium cordatum* three days old viewed from the dorsal surface. The antero-lateral arms have grown out and the stomodæum has opened into the œsophagus. The aboral spike and its skeleton are fully formed. *cil.* Posterior tuft of cilia at the apex of the aboral spike.

Fig. 11.—Larva of *Echinocardium cordatum* four days old, viewed from the dorsal surface. *l. p. c.* Backward growth of the cœlom on the left side of the stomach, which will be cut off as the left posterior cœlom. *r. p. c.* Similar growth of the cœlom on the right side of the stomach which will be cut off as the right posterior cœlom. *mad.* Left madreporic pore. *mad.*¹ Right madreporic pore. *p. d.* Rudiment of the skeleton of the postero-dorsal arm.

PLATE 15.

[All the following figures represent larvæ developing from the eggs of *Echinocardium cordatum* which have been fertilised with the sperm of *Echinus esculentus*.]

Fig. 12.—Hybrid blastula twenty-four hours old. *mes.* Primary mesenchyme.

Fig. 13.—Hybrid gastrula two days old. *cil.* Anterior tuft of cilia.

Fig. 14.—Hybrid gastrula (from another culture) two days old. *cæ.* Rudiment of cœlom. *calc.* Rudiment of skeleton. *mes.* Secondary mesenchyme.

Fig. 15.—Hybrid larva four days old viewed from the dorsal side. *a.l.* Antero-lateral rod of skeleton. *b.r.* Body-rod. *cil.* Anterior tuft of cilia now incorporated with longitudinal ciliated band. *cæs.* Larval œsophagus not yet joined to stomodæum. *r.r.* Vestigial recurrent rod.

Fig. 16.—Hybrid larva four days old, more advanced in development than that shown in fig. 15; viewed from the dorsal side.

Fig. 17.—Hybrid larva five days old viewed from the dorsal surface. *c.r.* In-bent crook at aboral end of body-rod.

Fig. 18.—Hybrid larva five days old viewed from the left side. *ad.* Ridge of thickened epithelium in the œsophagus carrying the aboral ciliated band. *an. anns.* *h.r.* Horizontal branch of the skeleton.

Fig. 19.—Hybrid larva six days old viewed from the dorsal surface. *r.r.* Recurrent rod.

Fig. 20.—Hybrid larva seven days old viewed from the dorsal surface. Notice that one antero-lateral arm has been absorbed.

Fig. 21.—Hybrid larva eight days old viewed from the dorsal surface. *mad., mad.*¹ The two madreporic pores.

Fig. 22.—Hybrid larva eight days old viewed from the ventral surface. *an.* Anus. *h.r.* Horizontal branch of larval skeleton.

Fig. 23.—Hybrid larva eight days old viewed from the left side. This is the only hybrid in which the aboral spike and its skeleton have been developed. Notice the distortion of the larva. It is as if the left antero-lateral arm and the left side of the oral lobe had been forcibly twisted away from the spectator. *b.r.* The right and left body-rods crossing each other. *cil.* Posterior tuft of cilia.